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PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF *AMPELOCISSUS LATIFOLIA* (ROXB.) PLANCH TUBEROUS ROOT USING UV-VIS, FTIR AND GC-MS

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ABSTRACT: The present investigation was carried to determine UV-VIS, FTIR and GC-MS analysis of *Ampelocissus latifolia* tuberous root. The ethanol extract was examined under visible and UV light for the proximate analysis. The UV-VIS profile of *Ampelocissus latifolia* tuberous root ethanol extract showed the peaks at 409 nm, 424 nm and 479 nm with the absorption of 1.682, 1.704 and 1.479 respectively. The FTIR spectrum was revealed the presence of alcohols, aromatic compound, alkanes, aldehydes, ketones, alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halides in ethanol extracts of *Ampelocissus latifolia* tuberous root. GC-MS analysis of ethanol extract of *Ampelocissus latifolia* tuberous root was revealed the presence of 14 phytochemical compounds. The presence of various functional groups and phytocompounds in *Ampelocissus latifolia* tuberous root confirm that it act as a most important source of drugs against various ailments.

INTRODUCTION: Medicinal plants regarded as one of the main source of drug for the health of individual and communities [1]. The information about medicinal properties of plant has been obtained on the basis of various medicinal systems such as Ayurveda, Unani and Siddha during many centuries [2]. All the existing medicinal plants considered as main source for the invention of new drugs beneficial to human beings [3]. Phytoconstituents are the natural bioactive compounds found within plants. These phytoconstituents along with nutrients form integral defense system to protect against various diseases [4].

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During last few decades UV, FTIR and GC-MS were acted as powerful techniques for the identification, separation and structural determination of phytochemicals[5]. Phytoconstituents determination is easily performed by using gas chromatography (GC) and liquid chromatography (LC) technique with definite revealing system. Gas chromatography-mass spectroscopy (GC-MS) helped in identification of compounds at less than 1 mg [6]. Commonly GC-MS applied for drug detection, environmental investigation and detection of unknown samples. GC-MS technique has been founds very effective for the separation and recognition of composite mixtures of phytochemicals [7].

Determination of chemical properties of phytocompounds present in plant provides necessary information about different functional groups revealing its medicinal properties [8]. The FTIR analysis produced spectrum in which

vibration of bond within chemical functional groups measure hence regarded as biochemical fingerprint of the sample [9]. FTIR spectroscopy is known as time-saving process to distinguish and identified functional groups [10].

Spectroscopic methods such as UV-Visible and FTIR can be used together or separately as conventional methods to detect phytoconstituents [11]. Hence, the objective of the present study is to identify the phytochemical constituents of *Ampelocissus latifolia* by using UV-VIS, FTIR and GC-MS technique.

MATERIAL AND METHODS:

Collection of plant material:

The plant material of *Ampelocissus latifolia* was collected during September- October 2013 from Wasali forest area of Buldhana District, Maharashtra, India. Plant was identified by using various floras. Herbarium specimen of the plant was deposited at Department of Botany, Shri Shivaji Science and Arts College Chikhli. Tuberous roots were collected, thoroughly washed with water to remove foreign matter; shade dried and then grinds into fine powdered by using mechanical grinder.

Extraction of plant sample:

The grinded, fine powdered of tuberous root was subjected to extraction by using soxhlet apparatus. About 20 gm of tuberous root powdered was successively extracted with ethanol for 8 hrs. Ethanol extract was filtered through Whatman No. 1 filter paper and the filtrate was collected (crude extracts). Ethanol extract was concentrated, solidified and used for further studies.

UV-VIS Spectroscopic analysis:

Ethanol extract of *Ampelocissus latifolia* tuberous root was examined under UV and Visible light for immediate investigation. Such ethanol extract of tuberous root was centrifuged at 3000 rpm for 10 minutes and filtered through filter paper (Whatman No.1) under high pressure of vacuum pump. The sample was diluted to 1:10 by using same solvents. The extract scanned in the wavelength range from 190-1100 nm using EQUIPS- TRONICS (EQ-826) Spectrophotometer and the peaks were detected.

FTIR Spectroscopic analysis:

Dried powdered of ethanol extract of *Ampelocissus latifolia* tuberous root was used for FTIR analysis. The dried 10 mg of extract powdered was mixed with KBr salt and encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The infrared spectrum of solid was recorded in the scan range from 4400-450 cm^{-1} on a FTIR spectrophotometer, Perkin Elmer Spectrum (RX1) with a resolution of $1cm^{-1}$. Perkin Elmer spectrophotometer was used to detect characteristic peak and their functional group.

GC-MS analysis:

The composition of ethanol extract of *Ampelocissus latifolia* tuberous root was analyzed by using GC-MS instrument (AccuTOF GCV). The GC-MS system was equipped with a FID detector and capillary column of HP-1 (30 m x 0.25 mm; film thickness 0.25 μm). Helium was used as carrier gas at a flow rate of 1ml/min and a split injector (split ratio 1:10). The oven temperature was programmed from 80-260^0 C at the rate of 10ºC/min and held at this temperature for 3 minutes and then increased to 280ºC at 5ºC/minute and held at this temperature for 9 min. The injector and detector temperatures were set at 250^0 C and 280^0 C respectively. Ethanol extract sample (0.1 μl) was injected into GC-MS instrument for its analysis. Ion source temperatures were maintained at 200^0 C and the mass spectra were taken at 70eV.

Identification of Components:

Interpretation of mass spectrum GC-MS was conducted using data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. The name, molecular weight, molecular formula and the structure of the components of *Ampelocissus latifolia* tuberous root were recorded.

RESULTS AND DISCUSSION:

The qualitative UV-VIS spectrum profile was selected for ethanol extract of *Ampelocissus latifolia* tuberous root powdered at a wavelength of 300 to 500 nm due to sharpness of the peak and proper baseline. The profile was shown peak at 409

nm, 424 nm and 479 nm with the absorption of 1.682, 1.704 and 1.479 respectively (**Table 1**).

TABLE 1: UV-VIS PEAK VALUES OF ETHANOL EXTRACT OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT

Sr. No.	Wavelength (nm)	Absorption value
1	409	1.682
2	424	1.704
3	479	1.479

The UV-VIS spectrum profile of ethanol extract of *Ampelocissus latifolia* tuberous root was shown in **Fig. 1.**

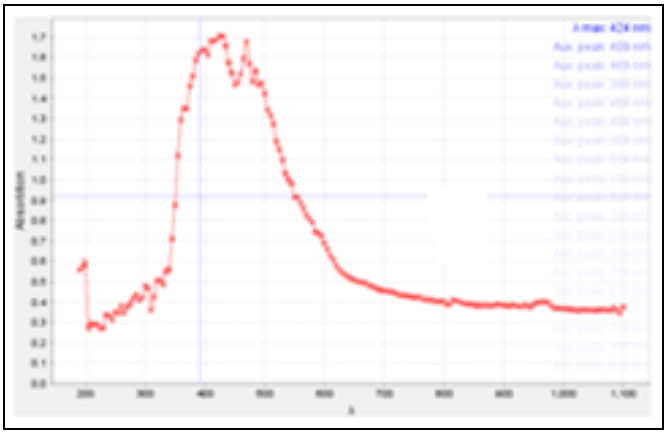


FIG. 1: UV-VIS SPECTRUM OF ETHANOL EXTRACT OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT.

The FTIR spectrum was used to identify the functional group of different phytoconstituents based on the peak values in an infrared region of ethanol extract of *Ampelocissus latifolia* tuberous root powdered. The result of FTIR peak values were shown in **Fig. 2.**

The FTIR spectroscopic analysis of *Ampelocissus latifolia* was revealed the presence of alcohols, aromatic compound, alkanes, aldehydes, ketones, alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halides in ethanol extracts of tuberous root powdered as shown in **Table 2**.

Pednekar and Raman, (2013) during FT-IR analysis of *Ampelocissus latifolia* (Roxb.) leaves was revealed that leaf powder showed alkynes, alcohols, esters, nitro compound, phenols, polysulfides and aliphatic iodo compounds. The stem powder showed alcohols, esters, aldehydes, ketones, phenols and nitro compound.

Raman *et al*., (2014) was reported the presence of alcohols, phenols, alkanes, aldehydes, alkenes, ethers, carboxylic acids, esters, alkyl halides, amines, amides, ketones, peroxides, etc. as functional groups during Fourier Transform Infrared (FT-IR) Spectroscopic study of *Ampelocissus latifolia* (Roxb.) Planch and it's different leaf extracts.

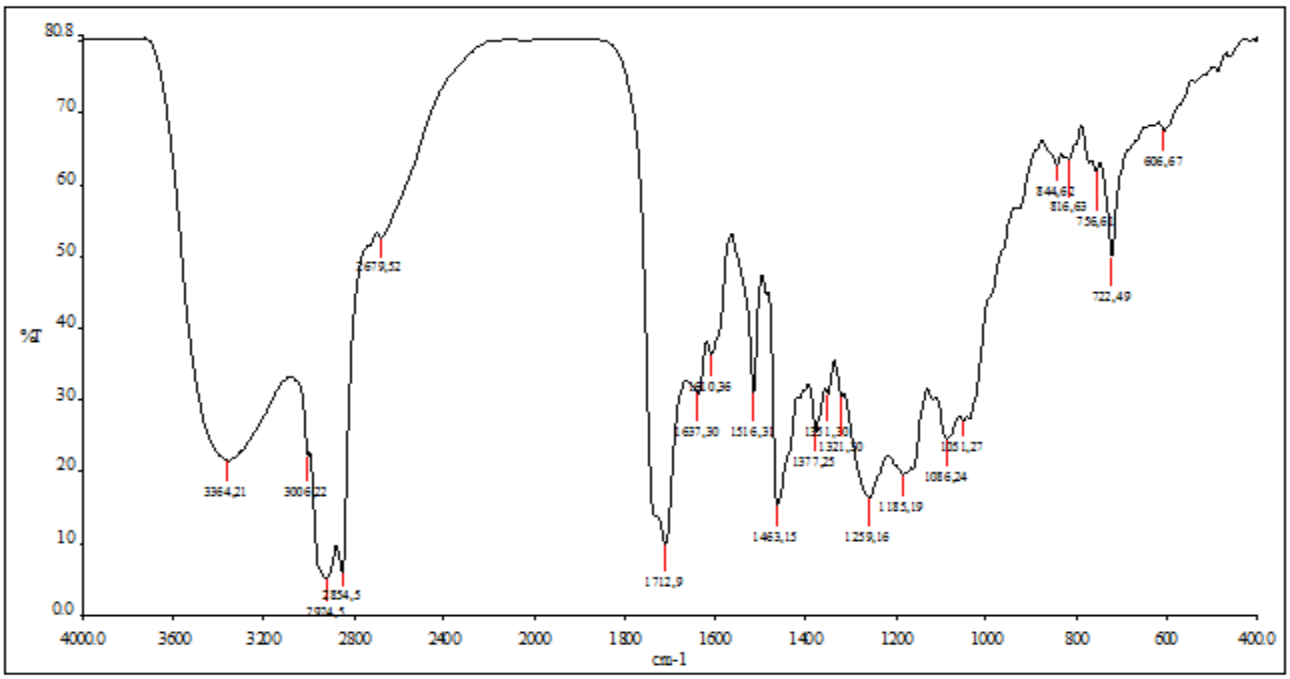
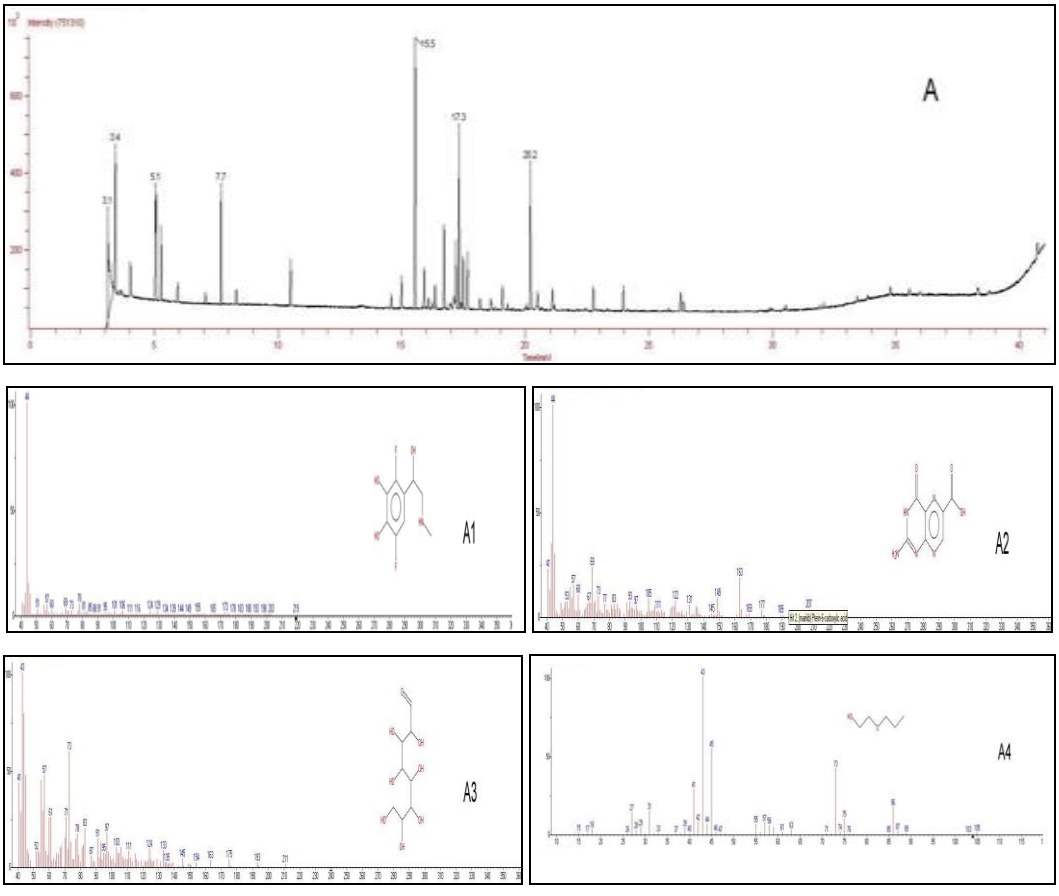


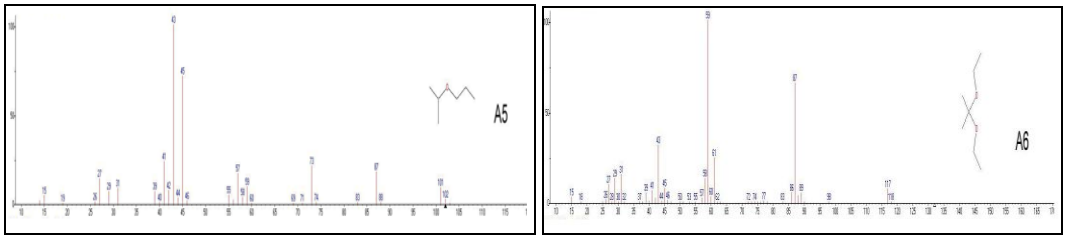
FIG. 2: FTIR SPECTRUM OF ETHANOL EXTRACT OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT

FIG. 2: FTIR SPECTRUM OF ETHANOL EXTRACT OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT.

Sr. No.	Wave Number Cm^{-1}	Bond Group Assignment	Functional	Group Frequency Cm^{-1}
1	3364.21	O-H stretch	H-bonded alcohols	3200-3600
2	3006.22	-C-H stretch	Aromatic compound	3000-3150
3	2924.5	C-H stretch	alkanes	2850-2970
4	2854.5	C-H stretch	alkanes	2850-2970
5	2679.52	O-H stretch	H-bonded carboxylic acid	2400-3400
6	1712.9	C=O stretch	ketones	1705-1725
7	1637.30	C=C stretch	alkenes	1600-1680
8	1610.36	C=C stretch	alkenes	1610-1680
9	1516.31	N-O asymmetric stretch	nitro compounds	1475-1550
10	1463.15	C-H bend	alkanes	1340-1470
11	1377.25	C-H bend	alkanes	1340-1470
12	1351.30	C-H bend	alkanes	1340-1470
13	1321.30	N-O asymmetric Stretch	nitro compounds	1300-1370
14	1259.16	C-N stretch	amines, amides	1180-1360
15	1185.19	C-N stretch	amines, amides	1180-1360
16	1086.24	C-F stretch	alkyl halides	1000-1150
17	1051.27	C-O stretch	alcohols, carboxylic acids, ethers, esters	1000-1320
18	844.62	C-H bend	alkenes	650-1000
19	816.63	C-H bend	alkenes	650-1000
20	756.61	C-H bend	alkenes	650-1000
21	722.49	C-H bend	alkanes	720-725
22	606.67	C-I bend	aliphatic ido compound	500-667

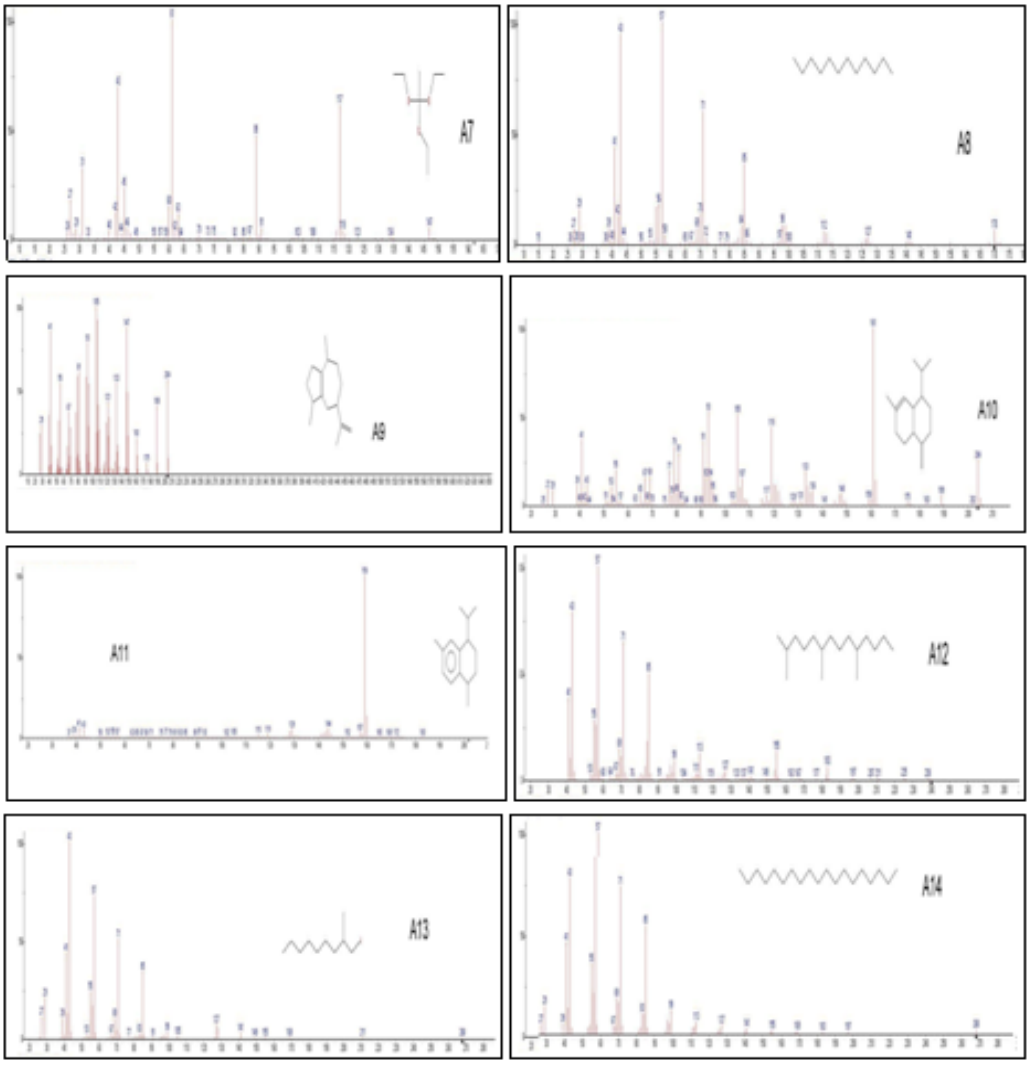
The GC-MS analysis of ethanol extract of *Ampelocissus latifolia* tuberous root was revealed the presence of 14 phytochemical compounds that could contribute to the medicinal property of the plant. The GC-MS chromatogram and mass spectrum of phytochemical compounds obtained in ethanol extract of *Ampelocissus latifolia* tuberous root were shown in **Fig. 3** and **4**.





A: GC-MS chromatogram of ethanolic extract of *Ampelocissus latifolia* tuberous root, **A1:** Mass spectra of Benzeneethanamine, 2, 5-difluoro-β,3, 4-trihydroxy-N-methyl-, **A2:** Mass spectra of Pterin-6-carboxylic acid. **A3:** Mass spectra of I- Gala-I-ido-octose, **A4:** Mass spectra of Ethanol, 2-propoxy, **A5:** Mass spectra of Propane, 1-(1-methylethoxy)-, **A6:** Mass spectra of Propane, 2, 2- diethoxy-.

FIG. 3: GC-MS CHROMATOGRAM AND MASS SPECTRUMS OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT



A7: Mass spectra of Ethane, 1, 1, 1-triethoxy-, **A8:** Mass spectra of Dodecane, **A9:** Mass spectra of Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octohydro-1, 4-dimethyl-7-(1-methylethenyl)-, [1S- (1∝, 4∝, 7∝)]-,**A10:** Mass spectra of Naphthalene, 1, 2, 3, 4, 4a, 5, 6,8a-octahydro-7-methyl-4-methyethyl)-, (1∝, 4a∝, 8a∝) –, **A11:** Mass spectra of Naphthalene, 1, 2, 3, 4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1S-cis)-,**A12:** Mass spectra of Tetradecane, 2, 6, 10-trimethyl-, **A13:** Mass spectra of 1-Iodo-2-methylnonane, **A14:** Mass spectra of Nonadecane.

FIG.4: MASS SPECTRUMSOFPHY TO CHEMICAL COMPOUNDS OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT

The identification of phytochemical compounds were confirmed using data base of National Institute Standard and Technology (NIST) and their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area in percentage (%)represented in **Table 3**. The first compound identified with less retention time (3.1min) was Benzeneethanamine, 2, 5-difluoro-β,3, 4-trihydroxy-N-methyl- whereas Nonadecane was the last compound which took longest retention time (26.3 min). The major phytoconstituents present in ethanol extract of tuberous root were Benzeneethanamine, 2, 5-difluoro-β,3, 4-trihydroxy-N-methyl-(31.21%), Pterin-6-carboxylic acid (31.21%), I- Gala-I-ido-octose(31.21%), Ethanol, 2-propoxy(0.53%), Propane, 1-(1-methylethoxy)- (0.53%), Propane, 2, 2- diethoxy-(0.84%), Ethane, 1, 1, 1-triethoxy-(0.84%), Dodecane(0.47%), Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octohydro-1, 4-dimethyl-7-(1-methylethenyl)-,[1S- (1∝, 4∝, 7∝)]-(0.18%), Naphthalene, 1, 2, 3, 4, 4a, 5, 6,8a- octahydro-7-methyl-4-methyethyl)-, (1∝, 4a∝, 8a∝) –(0.62%), Naphthalene, 1, 2, 3, 4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1S-cis)- (0.87%) , Tetradecane, 2, 6, 10-trimethyl-(0.48%), 1-Iodo-2-methylnonane(0.48%) and Nonadecane (0.48%) as listed in **Table 3**.

TABLE 3: GC-MS ANALYSIS OF *AMPELOCISSUS LATIFOLIA* TUBEROUS ROOT

Sr. No.	R.T.	Name of Compound	M.F.	M.W.	Peak Area %
A1	3.1	Benzeneethanamine, 2, 5-difluoro-β,3, 4-trihydroxy-N-methyl-	$C_9H_{11}O_3F_2N$	219	31.21
A2	3.1	terin-6-carboxylic acid	$C_7H_5N_5O_3$	207	31.21
A3	3.1	I- Gala-I-ido-octose	$C_8H_{16}O_8$	240	31.21
A4	4.0	Ethanol, 2-propoxy	$C_5H_{12}O_2$	104	0.53
A5	4.0	Propane, 1-(1 methylethoxy)-	$C_6H_{14}O$	102	0.53
A6	5.3	Propane, 2, 2- diethoxy-	$C_7H_{16}O_2$	132	0.84
A7	5.3	Ethane, 1, 1, 1-triethoxy-	$C_8H_{18}O_3$	162	0.84
A8	10.5	Dodecane	$C_{12}H_{26}$	170	0.47
A9	15.9	Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octohydro-1, 4-dimethyl-7-(1-methylethenyl)-, [1S- (1∝, 4∝, 7∝)]-	$C_{15}H_{24}$	204	0.18
A10	16.7	Naphthalene, 1, 2, 3, 4, 4a, 5, 6,8a- octahydro-7-methyl- 4-methylene-1-(1-methylethyl)-, (1∝, 4a∝, 8a∝) -	$C_{15}H_{24}$	204	0.62
A11	17.7	Naphthalene, 1, 2, 3, 4-tetrahydro-1, 6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{22}$	202	0.87
A12	22.7	Tetradecane, 2, 6, 10-trimethyl-	$C_{17}H_{36}$	240	0.48
A13	22.7	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	0.48
A14	26.3	Nonadecane	$C_{19}H_{40}$	268	0.48

Where, R.T. = Retention time, M.F. = Molecular formula, M.W. = Molecular weight.

CONCLUSION: UV spectrophotometric analysis is a simple, rapid and accurate method for the determination of phytoconstituents present in crude drug powdered of medicinal plants. The FTIR spectral analysis has shown the presence of characteristic functional groups in ethanol extracts of *Ampelocissus latifolia* plants. GC-MS analysis has revealed the presence of different active compounds of important medicinal value against various ailments in studied plants. Hence study revealed the isolation, characterization, purification and biological activity of specific active compounds are necessary for their further studies.

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